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ORIGINAL ARTICLE

ANTIBODY RESPONSE AGAINST HEPATITIS B VIRUS AFTER VACCINATION AND SEROPREVALENCE OF HBV IN CHILDREN IN ADDIS ABABA, ETHIOPIA

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ABSTRACT

Background: Around two billion people have been infected with HBV worldwide, and more than 240 million are chronic carriers. Vaccine introduction for HBV in children was officially launched by the WHO in 1980. Since then the vaccine response level has been determined in different countries. Since the introduction of the vaccine in Ethiopia in 2007, few studies have been conducted to assess the antibody response against the HBV vaccine.

Objectives: The aim of this study is to determine antibody response against HBV after hepatitis B vaccination and assess the seroprevalence of HBV in children in Addis Ababa, Ethiopia.

Methods: A cross-sectional study was conducted using a multistage probability sampling technique. Four hundred and fifty children between the ages of 5 and 8 living in Addis Ababa were enrolled. Socio-demographic characteristics were obtained through a structured questionnaire and three to four ml of blood was collected from each child. ELISA was performed to determine antibody levels against HBV.

Results: The mean age was 7₊₁ (SD) years. Anti-HBs were detected in 54.3% (208/450) of children with a slightly higher proportion of protective level in females 98 (54.7%) than males 110 (53.9%). The overall vaccine coverage in our study was 85.1%. The proportion of children with a protective level (>10 mIU/ml anti-HBs antibody) declined as the age of the child increased: 52.6%, 60%, 43.5% and 37.1% at the age of 5, 6, 7 and 8 years, respectively. Seroprevalence of HBsAg was 0.4%, whereas seroprevalence of anti-HBc was 5.6%. Age was negatively correlated with the response level (p=0.001), whereas sex and history of HBV infection had no significant association. Age was also significantly associated with seroprevalence of anti-HBc (p=0.003).

Conclusion: The HBV vaccine coverage in children was high but antibody response against the vaccine appears low. Seroprevalence of the virus was also low. The low response level to the vaccine should be a concern and re-vaccination or booster doses should be considered for non-responding children. Further studies should also be undertaken.

Keywords: HBV, antibody response level, vaccination status, HBsAg, Anti HBsAg, Anti HBc

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global health concern. Around two billion people have been infected with HBV worldwide, and more than 257 million people are currently living with hepatitis B virus infection (1). There are an estimated 600,000 deaths annually from complications of HBV-related liver diseases (2). The highest numbers of HBsAg carriers are found in developing countries with limited medical facilities. Endemicity levels of chronic HBV infection are classified as high (>8%), intermediate (2-8%), or low (<2%). Based on this classification, sub-Saharan African countries including Ethiopia are considered regions of high endemicity (1).

HBV infection can be prevented by using the HBV vaccine. HBV vaccine has been commercially available since the 1980s. In 1991, WHO recommended the integration of the HBV vaccine into the national immunization programs and by the end of 2005, the vaccine coverage was 82.3% globally (3, 4). Hepatitis B vaccine for infants had been introduced in 183 countries by the end of 2013. In 2007, almost all sub-Saharan African countries had Hepatitis B vaccination in their national program (5). HBV vaccine was introduced into the Ethiopian Expanded Program of Immunization (EPI) in 2007 and national coverage had reached 86% by 2015 (6).

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Immune response to the vaccine can be determined by measuring the concentration of antibodies against the HBsAg. Anti-HBs in vaccinated children decline with time, especially during the first few years of vaccination. Most children produce a high concentration of antibodies following vaccination; however, few children can have low or no response to the vaccine. The reason for this low or no response against the HBV vaccine is not well known. However, the site of injection and modes of administration are thought to be critical factors in achieving an optimal response (7). Data on the level of immune responses against HBV vaccine in Ethiopia is very limited. This study was conducted to contribute to the baseline data needed for further monitoring of HBV vaccination effectiveness in Ethiopia and provides information on the level of immune responses against HBV vaccine among children in Addis Ababa.

PATIENTS AND METHODS

Study area and period: The study was conducted in Addis Ababa, which is the capital city of Ethiopia from April 2016 to May 2017. A total number of 450 children (5-8 years old) were recruited.

Study design: A prospective cross-sectional study design was used. Healthy appearing 5-8 years old children were included in the study following their parents' consent. Vaccination status was used to include and exclude participants.

Sample size determination and sampling technique: The required sample size for this study was calculated using 50% of prevalence in vaccine response using the following formula :

$$n = \frac{\left(\frac{Z\alpha}{2}\right)^2 pq}{d^2}$$

where, n = sample size

Z = confidence interval

q = 1 - p p = proportion

Using confidence interval of 95%, Z=1.96

$$\begin{aligned} N &= \frac{(1.96)^2 * 0.5 * 0.5}{(0.5)^2} \\ &= 385 \end{aligned}$$

The total number of the samples with 10% non-respondents should come to 424. However, just to be safe we collected a total of 450 samples.

Data collection: There was a structured questionnaire to collect all relevant information about the study participants. After the participant's family agreed to take part in the study, they signed an informed consent form. 3-4 ml of blood sample was collected from each child and the serum was separated and stored at -20°C until further use.

Ethical clearance: Ethical clearance was obtained from the AHRI/ALERT Research Ethics Review Committee and the department of ethical research committee. A support letter was obtained from the Addis Ababa Health Bureau and from the health bureaus of each sub city. Written informed consent was obtained from each participant's parent or guardian before enrollment.

Data analysis and interpretation: SPSS Software statistical package version 20.0 was used to analyze the data. Association was determined by Chi-square test. P-values less than 0.05 were considered as statistically significant.

Serological assays: Serum level HBsAg, anti-HBc, and anti-HBsAg were determined using sandwich ELISA, where antigens/monoclonal antibodies were used both for capture and detection. All ELISA experiments were performed using BIO-RAD, Monolisa ELISA test kits, France. The test kits have a high sensitivity and specificity and each test procedure was undertaken according to the manufacturer's instruction based on standard operating procedures.

Operational definitions

Hepatitis B virus:- A DNA virus that attacks the liver and can cause both acute and chronic disease

HBsAg:- It is the surface antigen of the hepatitis B virus. It indicates the presence of active hepatitis B infection.

Anti-HBsAg:- An antibody which is produced against HBV infection or vaccination.

Anti-HBcAg:- An antibody that indicates previous or ongoing infection with the hepatitis B virus in an undefined time frame.

RESULTS

Socio-demographic characteristics: The mean age of the study participants was 7±1 (SD) years. Among these, 244 (54.2%) were male and 383 (85.1%) had been vaccinated. Forty-nine (10.9%) children had a history of infectious disease, fifteen (3.3%) had a history of noninfectious disease, while 3(0.7%) were HIV positive (Table 1).

Table1: Socio demographic characteristics of children between the age of 5 and 8 years old in Addis Ababa Ethiopia.

Characteristics	Categories	Number (n)	Percent (%)
Sex	Male	204	54.2
	Female	206	45.8
Age	5	95	21.1
	6	130	28.9
	7	124	27.6
	8	101	22.4
Place of birth	Urban	422	93.8
	Rural	28	6.2
Sub city	Gulele	45	10
	Kirkos	92	20.4
	Lideta	313	69.5
History of previous disease status	Yes	66	14.7
	No	384	85.3
Disease type	Infectious	49	10.9
	Non Infectious	15	3.3
	Immunocompromised	3	0.7
Vaccination status	None	383	85.1
	Vaccinated for HBV	383	85.1
	Non vaccinated for HBV	67	14.9

Table 2: The coverage of hepatitis B vaccination among 5-8 year-old children in Addis Ababa, 2016-2017.

Characteristics	Categories	Number (n)	Percent (%)
Sex	Male	204	54.2
	Female	206	45.8
Age	5	95	21.1
	6	130	28.9
	7	124	27.6
	8	101	22.4
Place of birth	Urban	422	93.8
	Rural	28	6.2
Sub city	Gulele	45	10
	Kirkos	92	20.4
	Lideta	313	69.5
History of previous disease status	Yes	66	14.7
	No	384	85.3
Vaccination status	Vaccinated for HBV	383	85.1
	Not vaccinated for HBV	67	14.9

Status	Male		Female		Total		P value
	Number (n)	Percent (%)	Number (n)	Percent (%)	Number (n)	Percent (%)	
Vaccinated	204	83.6	179	86.9	383	85.1	0.329
Non vaccinated	40	16.4	27	13.1	67	14.9	
Total	244	100	206	100	450	100	

Vaccination status: 383 (85.1%) out of 450 children had received HBV vaccination, of which 99.2% (380/383) of them had received the complete three doses of the vaccination. Only 2 (0.8%) children among the vaccinated had taken just two doses of the vaccine. There was no one who took only a single dose of the vaccine. The proportion of vaccinated female children (86.9 %) was slightly more than that of their male counterparts (83.6 %) (Table 2).

Concentration of Anti HBsAg: Anti-HBsAg concentration > 10mIU/ml was observed in 214 (47.6%) children, 6 of whom were unvaccinated. Among the 383 vaccinated children, 208 (54.3%) had a protective level of antibody concentration (anti-HBsAg concentration of > 10mIU/ml), while the remaining 47.3% did not. Among 67 unvaccinated children, 61 (91%) had anti HBsAg < 10mIU/ml. From the three HIV infected participants, two of them were vaccinated. However, they did not have protective level antibody response (anti-HBsAg < 10mIU/ml).

The distribution of the antibody levels against Hepatitis B vaccine is shown in Figure 1.

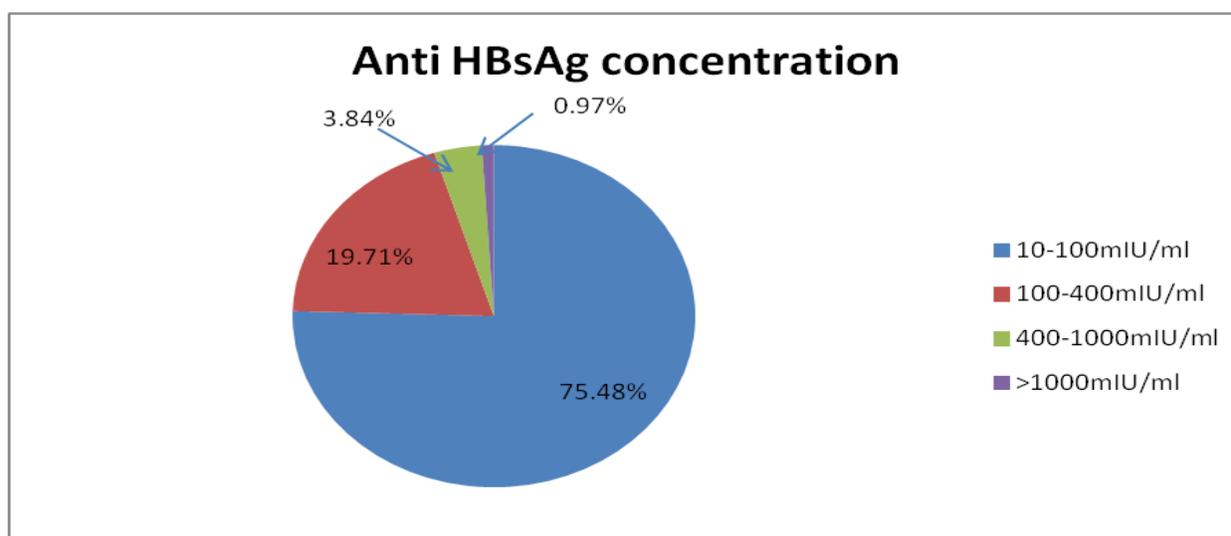


Figure 1: Antibody response against Hepatitis B vaccine in children between 5-8 years, Addis Ababa Ethiopia

Table 3: Sero-prevalence of anti-HBc in 5-8 years old children in Addis Ababa, 2016-2017

Variables	Categories	Number (n)	Percent (%)	P value
Sex	Male	15(244)	6.1	0.551
	Female	10(196)	4.9	
Vaccination status	Vaccinated	10(383)	2.6	0.000
	Not vaccinated	15(67)	22.4	
Age	5	4(95)	4.2	0.03
	6	3(130)	2.3	
	7	5(124)	4.0	
	8	13(101)	12.9	
Previous disease status	Yes	5(66)	7.6	0.438
	No	20(384)	5.2	

Anti-HBsAg concentration by age: A protective level antibody response against HBV vaccine was observed in 52.6%, 60%, 43.5% and 37.1% of children at the ages of 5, 6, 7, and 8, respectively. There was a significant association between age and the concentration of anti-HBsAg ($p=0.001$). The levels of antibody concentration decreased as the age of the participant increased.

Seroprevalence of hepatitis B: From a total of 450 children, only two (0.4%) were positive for HBsAg and 25 (5.6%) were positive for anti-HBc. 1 child (0.2%) was positive for both HBsAg and anti-HBc (Table 3). The two children who were positive for HBsAg were females, 5 years old, asymptomatic, and vaccinated for HBV.

Among anti-HBc positive children, 15 (6.1%) were male while 10 (4.9%) were female. There was no significant association between sex and anti-HBcAb ($p = 0.551$). Ten (2.6%) of anti-HBc positive children had received vaccination prior to this study. Among non-vaccinated children in this study, 15 (22.4%) were anti-HBc positive. There was a negative correlation between vaccination status and anti-HBc positivity ($p=0.000$).

The seroprevalence of anti-HBc was 4.2%, 2.3%, 4% and 12.9% in 5, 6, 7 and 8 year old children, respectively. Age was significantly associated with anti-HBc seroprevalence ($p=0.03$).

DISCUSSION

The primary goal of vaccination against HBV is to generate an effective antibody response against the virus. The efficacy of HBV vaccine has not been determined in Ethiopia since its introduction in 2007. HBV vaccine coverage rate observed (85.1%) in our study is comparable with the estimated (86%) national coverage reported in 2015 (6). Evidence has shown that the immune response against the vaccine decreases with increasing age. Our study observed a similar association between age and immune response against HBV vaccine.

In this study, 54.3% of vaccinated children had protective antibody response, as well as was obtained with low seroprevalence of HBsAg and anti-HBc of 0.4% and 5.6%, respectively. This result is comparable with a study conducted in Yemen and in Iran, where 54.8% and 56.3% of the children had protective antibody responses, respectively (8, 9).

However, in other areas, higher proportions of children were reported with protective level antibody responses in comparison to our study. Studies conducted in different areas of Iran observed that 78% of 5-10 years old children (10), 84% of 5 to 7 years old children (11), and 87.6% of under 7 years old children (12) had a protective level antibody response against HBV. A study that was conducted in Spain also revealed that 85% of children at the age of seven had protective level antibody response to the vaccine (13). The difference in these countries could be attributed to differences in dose, vaccine type, and vaccination route.

In contrast to our study, a lower proportion of children with protective level antibody responses were observed in other studies. For example, in a study conducted in Egypt, 39.3% of the children (6-12 years old) had protective level antibody responses (14). In addition, in different areas of Iran, only 47.9% of 10-11 year old children (15), 48% of 7 to 9 year old children (16), and 30 % of the 8 months to 15 year old children (17) had between ages of 8 months and 15 years had protective level antibody responses against HBV vaccine. These differences may be due to sampling difference, type of vaccine, and different age for vaccine administration.

Seroprevalence of HBsAg among vaccinated children varies in different countries ranging from 0-2.5%. Seroprevalence of HBsAg was 1.8% in Yemen (8), 0.13% in Nepal (18), 2.3% in Papua New Guinea (19), 0.77% in Eastern China (20), 2.5% in northwest China (21), while it was 0.4% in our study. This difference in seroprevalence of HBV infection could be attributed to the difference in vaccine coverage and difference in vaccination schedule.

In our study, seroprevalence of anti-HBc was 5.6%, which is lower than anti-HBc seroprevalence observed in studies conducted in Gambia (17.7%) (22), China (14.1%) (21) and Iran (7.5%) (24). These discrepancies in anti-HBc seroprevalence could be attributed to age difference, race, prevalence of HBV, and immune response level.

Nowadays, mutant hepatitis B viruses are spreading globally. Vaccination regime and vaccine type should also be considered when we administer the vaccine to the child. High seroprevalence of anti-HBc in vaccinated children may indicate the presence of an occult HBV infection, which is a concern for everyone that needs to be addressed (25).

Limitations of the study

Some children did not come with their vaccination card. Therefore, we had to use the words of their parents/guardians as evidence for vaccination, which is not always reliable. Other serological markers of active HBV infection, like HBeAg, were not examined in this study. Further, there was an unequal number of vaccinated and non-vaccinated children and the study design did not account for occult infections.

Conclusion and Recommendation

The vaccine coverage observed in this study is similar to that of the national estimate in 2014. However, less than half of the children had a protective level of antibody response against HBV vaccine. Further, a negative association between anti-HBsAg antibody concentration and age was observed. Serological markers for hepatitis B virus were low: 0.4% for HBsAg and 5.6% of anti-HBc.

Persistence of anti-HBs antibodies is necessary for the long-term protection against hepatitis B virus infection. Even if different factors can contribute to low antibody response against the vaccine, we need to follow up children after vaccination, in order to see the effect of the vaccine in producing the desired response over time.

Finally, further studies should be undertaken to determine the duration of antibody response against HBV vaccine that may help in which years the vaccine response becomes less and less. For those who did not respond to the vaccine, booster doses should be given to enhance immunological responses to the vaccine. This can be important to elevate the vaccine response. Follow up is needed for those children who are administered with booster doses to evaluate response against the vaccine in those children.

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Competing of interest

There was no conflict of interest among the authors or with any other parties.

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